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The Brain Finger Protein Gene (*ZNF179*), a Member of the RING Finger Family, Maps Within the Smith-Magenis Syndrome Region at 17p11.2

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Smith-Magenis syndrome (SMS) is caused by a microdeletion of 17p11.2 and comprises developmental and growth delay, facial abnormalities, unusual behavior and sleep problems. This phenotype may be due to haploinsufficiency of several contiguous genes. The human brain finger protein gene (*ZNF179*), a member of the RING finger protein family, has been isolated and mapped to 17p11.2. FISH analyses of metaphase or interphase chromosomes of 6 patients with SMS show that *ZNF179* was deleted in one of the 2 homologs (17p11.2), indicating a possible association of the defect of this gene with the pathogenesis of SMS. Furthermore, using a prophase FISH ordering system, we sublocalized *ZNF179* proximally to *LLGL* which lies on the critical region for SMS. Am. J. Med. Genet. 69:320–324, 1997.

KEY WORDS: Smith-Magenis syndrome; 17p11.2; gene mapping; FISH; ZNF179; LLGL

INTRODUCTION

A region of 17p is associated with at least 5 distinct syndromes: Miller-Dieker syndrome (MDS), Charcot-Marie-Tooth disease type 1A (CMT1A), hereditary neuropathy with liability to pressure palsies (HNPP), Sjögren-Larsson syndrome (SLS), and Smith-Magenis syndrome (SMS). The MDS deletions have been mapped to 17p13.3 [Ledbetter et al., 1989]. CMT1A and HNPP are due to the reciprocal DNA duplication/deletion of a 1.5-Mb region of 17p11.2-p12 [Lupski et al., 1991; Raeymaekers et al., 1991; Pentao et al., 1992; Chance et al., 1993, 1994; Wise et al., 1993], which includes *PMP22,* the gene responsible for both disorders. In addition, based on a linkage analysis in SLS families of non-Swedish ethnic origins, SLS has been mapped to a limited region within 17p12 proximal to CMT1A [Rogers et al., 1995]. In contrast, an SMS microdeletion is present at more proximal in 17p11.2.

Patients with SMS show several nonspecific clinical manifestations such as mental retardation, facial abnormalities, and slow growth in addition to more specific manifestations such as unusual behavior and sleep problems [Smith et al., 1986; Greenberg et al., 1991]. Thus, SMS is considered a contiguous gene syndrome. So far 5 genes have been mapped to the SMS critical region: genes coding small RNA U3 (*snRNA U3*) [Chevillard et al., 1993], the human homolog of the Drosophila melanogaster flightless-1 (*FLI1*) [Chen et al., 1995], microfibril-associated glycoprotein (*MFAP4*) [Zhao et al., 1995], cytosolic serine hydroxymethyltransferase (*cSHMT*) [Elsea et al., 1995], and of the murine mgl-1 (*LLGL*) [Koyama et al., 1996].

The human, mouse and rat genes encoding brain finger protein (bfp), a member of RING finger protein family, have been isolated and localized by fluorescence *in situ* hybridization (FISH) [Matsuda et al., 1996]. Of these, the human BFP gene (*ZNF179*) was mapped to 17p11.2 [Matsuda et al., 1996]. The expression of *ZNF179*-mRNA was detected predominantly in the brain, and bfp proteins were also shown in both mouse and rat brain. To define the participation of this gene in SMS, we performed FISH experiments on metaphase and interphase chromosomes from 6 SMS patients whose karyotype analyses had shown microdeletions of 17p11.2. Furthermore, the precise sublocalization of

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this gene with respect to *LLGL* was determined by prophase FISH.

SUBJECTS, MATERIALS AND METHODS

SMS Patients

Six unrelated SMS patients examined in this study were described elsewhere [Kondo et al., 1991; Koyama et al., 1996]. All of them show visible microdeletions of sub-band p11.2 on either 17-homolog upon high-resolution banding. Four of these patients (KY, MT, MS and AS) lack *LLGL* in one copy of chromosome 17 [Koyama et al., 1996]. All 6 patients show the unusual behavior typical of SMS; details of the clinical manifestations of the 2 patients (KS and HN) were described by Kondo et al. [1991].

Mapping of *ZNF179* to Chromosomes From 6 SMS Patients

The 10-kb lambda phage clone, hgA, contains the sequences for RING finger domain of human brain finger protein [Matsuda et al., 1996]. The references for the positions of 17cen and 17p11.2 in multicolor FISH were *LLGL,* as well as CI17-321 and CI17-536 cosmids which are mapped to 17cen and 17p11.2, respectively [Inazawa et al., 1993]. The cosmids of *LLGL* and CI17-536 are localized within 17p11.2 microdeletions in the 6 patients [Koyama et al., 1996].

Chromosome slides were prepared from EB-virus-transformed lymphoblastoid cell lines from the 6 patients. FISH was carried out according to Inazawa et al. [1993]. In brief, DNA probes of hgA (*ZNFA179*) and CI17-321 were labeled with biotin-16-dUTP and digoxigenin-11-dUTP (both from Boehringer-Mannheim, Germany) by nicktranslation, respectively. The labeled probes were denatured at 75°C for 10 min and hybridized to the denatured chromosomes at a final concentration of 25 ng/μl in a mixture of 50% formamide, 10% dextran sulfate (Sigma, St Louis, USA), 2xSSC, sonicated salmon sperm DNA (2 μg/μl), *Escherichia coli* tRNA (2 μg/μl), and Cot-1 DNA (125 ng/μl). The hybridization signals were detected with FITC-avidin and antidigoxigenin rhodamine (both from Boehringer-Mannheim); chromosomes were then counterstained with DAPI (1 μg/ml), and mounted in an anti-fade solution containing 1% DABCO (Sigma). Three-color FISH with hgA, *LLGL* and CI17-536 was performed as in our previous report [Inazawa et al., 1992]. Using hgA double-labeled with biotin-16-dUTP and digoxigenin-11-dUTP, *ZNF179* was visualized as yellow pseudocolor signals generated by FITC and rhodamine. Multicolor signals were simultaneously observed using a Nikon FXA fluorescent microscope equipped with double or triple band-pass filters (Nikon, Tokyo, Japan).

Ordering of *ZNF179* and *LLGL* on 17p11.2 by Prophase FISH

To determine the physical order of *ZNF179* and *LLGL* on sub-band 17p11.2, we used a prophase FISH ordering system [Inazawa et al., 1994]. In brief, 0.5 μg of each *LLGL* cosmid and *ZNF179* lambda phage were labeled with biotin-16-dUTP and digoxigenin-11-dUTP, respectively. Elongated prophase chromosomes were prepared from cultured lymphocytes by means of thymidine synchronization and bromodeoxyuridine release in the presence of topoisomerase II inhibitor ICRF 154 [Inazawa et al., 1994]. Hybridization was performed at 37°C overnight on denatured-chromosome slides. After washing, the slides were incubated at 37°C with FITC-avidin and anti-digoxigenin rhodamine. The slides were washed at room temperature for 10 min each in 4xSSC, 4xSSC containing 0.05% Triton X-100, and 4xSSC, then counterstained with DAPI (1 μg/ml). To determine the order of *ZNF179* and *LLGL* probes by multi-color FISH, FITC and rhodamine signals were simultaneously detected through a triple band-pass filter, then the contours on DAPI-stained prophase chromosomes were delineated through a UV-2A filter (Nikon). The order and orientation of signals on sub-band 17p11.2 were determined by pairwise comparison along the long axis of chromatids.

RESULTS

FISH of *ZNF179* in 6 SMS Patients

More than 80 cells (over 30 metaphases and 50 interphases) were examined on each of the 6 patients. FITC signals specific for *ZNF179* were detected on only one copy of chromosome 17 of metaphase cells of all 6 patients, although rhodamine red signals specific for the centromere of chromosome 17 were detected on both homologs (Fig. 1a). In addition, three-color FISH with *ZNF179, LLGL* and CI17-536 detected only one spot of each at one of the 2 homologs in interphase nuclei from the 6 patients (Fig. 1b). These results indicated that one copy of *ZNF179* together with *LLGL* is lacking in these patients.

Ordering of *ZNF179* and *LLGL* by Prophase FISH

To determine the physical order of *ZNF179* and *LLGL,* we examined prophase chromosome 17 showing two different colored signals of a pair of *ZNF179* and *LLGL* cosmid probes. Relative positions of the signals were scored along the long axis of chromosomes. Of 20 prophase chromosomes examined, 17 had 17pter-green (FITC)-red (rhodamine)-17cen signals on both chromatids (Fig. 2) and FITC overlapped rhodamine signals on 17p11.2 in three. These results indicate that the physical order of *ZNF179* and *LLGL* is 17pter-*LLGL*-*ZNF179*-17cen.

DISCUSSION

We have shown that the human brain finger protein gene (*ZNF179*), a member of RING finger protein family, was deleted on one copy of chromosome 17 in all of the 6 SMS patients. SMS patients with an interstitial microdeletion of 17p11.2 have peripheral neuropathy, seizure, sleep disturbance, and behavior abnormalities including hyperactivity and self-mutilation [Smith et al., 1986; Greenberg et al., 1991], in addition to non-specific mental retardation, facial anomalies and developmental delay. These phenotypes suggest that the SMS critical region contains important gene(s) associ-

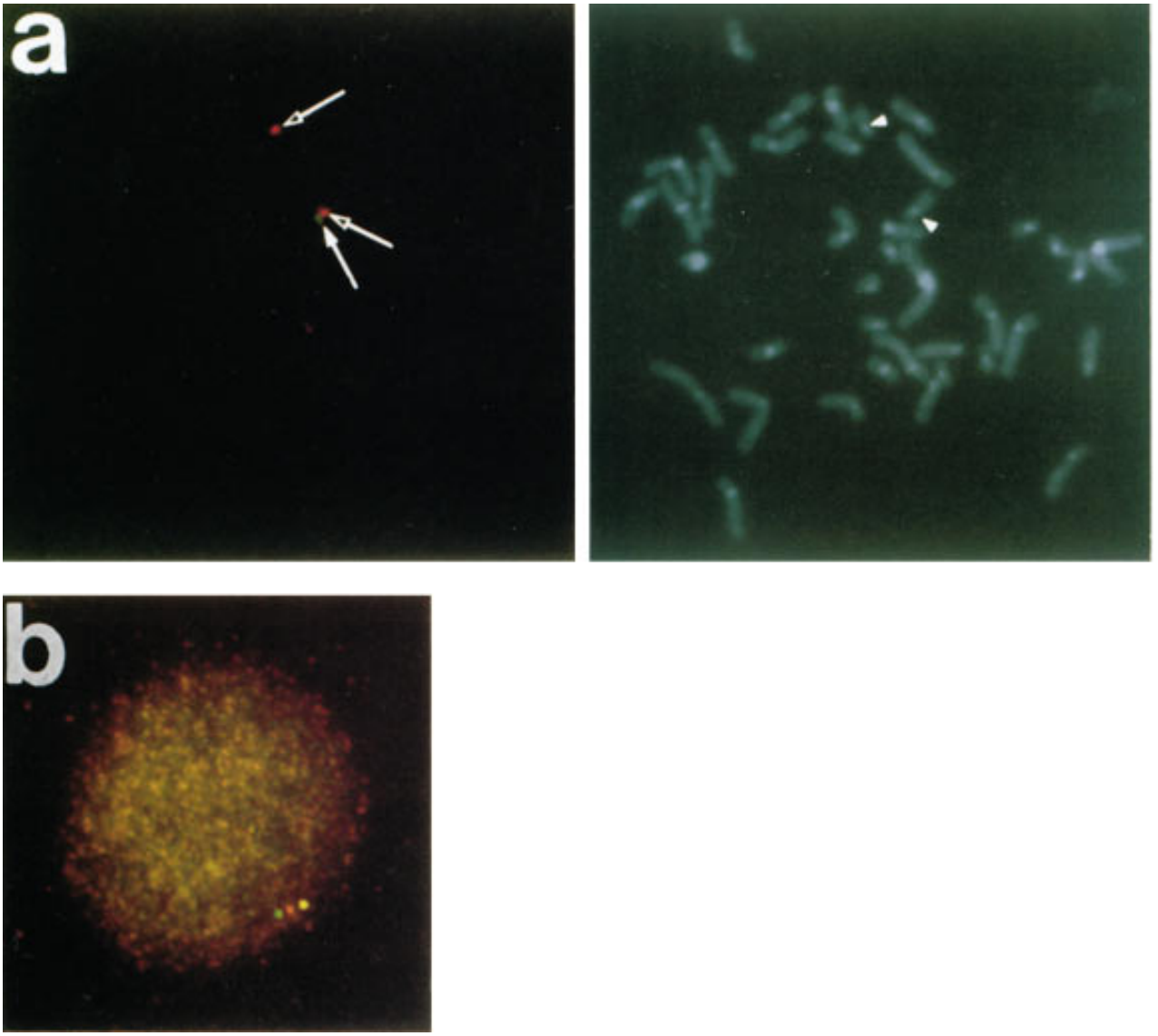

Fig. 1. FISH analysis in SMS patients. **a:** (Left) An FITC signal (arrow) specific for *ZNF179* was detected on one of two chromosome 17 homologs in an SMS patient (AS), although rhodamine red signals (open arrow) specific for 17cen were detected on both homologs. Right: The same metaphase chromosomes were counterstained with DAPI. Arrowhead indicates chromosomes 17. **b:** Representative nucleus from an SMS patient (HN) showing three-color FISH with probes of hgA (*ZNF179*), *LLGL* and CI17-536. Only one spot of each probe was detected on one chromosome 17p11.2, indicating that *ZNF179* (yellow) and *LLGL* (red) together with CI17-536 (green) are missing from one copy of chromosome 17 in this patient.

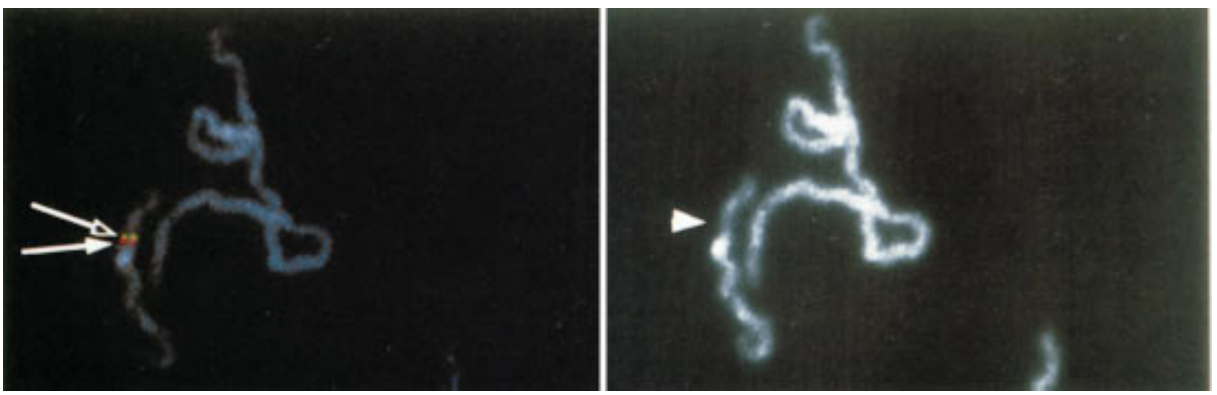

Fig. 2. Ordering of *ZNF179* and *LLGL* by prophase FISH. **Right:** A pair of FITC-labeled hgA (*ZNF179*) and rhodamine-labeled *LLGL* cosmid were detected in the order: 17pter-*LLGL*-*ZNF179*-17cen. **Left:** The same chromosomes were counterstained with DAPI.

ated with neuronal development, differentiation and function.

The brain finger protein (bfp) of the rat was the first to be isolated and characterized. The expression of bfp mRNA was predominantly detected in the brain, and bfp proteins were also shown in the rat and mouse brain. The bfp protein was not detected in untreated P19 embryonic carcinoma cells but was evident after exposure to retinoic acid when the cells differentiated into a neural cell-like form. These findings suggest that the bfp protein is up-regulated during the neural differentiation of P19 cells by retinoic acid and that it is a putative transcription regulator in the brain. Hence, human BFP may also be important in neuronal development and differentiation, and the haploinsufficiency of this gene might be involved in the pathogenesis of SMS.

Since a cytogenetically detectable deletion encompasses ⩾2–3 Mb [Ledbetter and Cavenee, 1989], it is likely that several genes lie on the SMS deleted region. Extensive studies of physical mapping spanning the SMS deleted region have been reported [Greenberg et al., 1991; Guzzetta, et al., 1992; Chevillard et al., 1993]. Chevillard et al. [1993] suggested that the SMS critical region is over 2–2.5 Mb, and variations of the sizes of microdeletions are likely to be due to the differences of the proximal breakpoints. Our study of prophase FISH showed that *ZNF179* is proximal to *LLGL* at 17p11.2. SMS shows phenotypic variations similar to those of other congenital chromosome abnormalities [Smith et al., 1986; Kondo et al., 1991; Greenberg et al., 1991; Zori et al., 1993; Finucane et al., 1993, 1994; Colley et al., 1993]. Thus, the presence or absence of *ZNF179* within the critical region for SMS should be examined in more SMS patients, and studies of biological function of this gene are required to determine if *ZNF179* is involved in eliciting the SMS-specific phenotypes.

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